

Appl. No. 10/538,840
Amendment dated: July 21, 2008
Reply to OA of: February 21, 2008

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claims 1-26(canceled).

27(currently amended). An isolated polynucleotide which encodes ~~codes~~ for a protein with trans-sialidase activity, wherein said polynucleotide ~~[[and]]~~ can be isolated from *Trypanosoma congoense* and which comprises ~~[[a]]~~ one of the nucleic acid sequence~~s~~ of sequences selected from the group consisting of SEQ ID NO: 1 ~~[[or]]~~ and 3; the polynucleotides complementary to the same; or ~~and~~ the nucleotide sequences derived differing from these said polynucleotides by degeneration of the genetic code.

28(currently amended). The isolated polynucleotide of claim 27, which encodes ~~codes~~ for a protein with trans-sialidase activity and which catalyzes the transfer of sialic acid from a donor onto an acceptor molecule.

29(currently amended). An isolated oligonucleotide, which hybridizes with a polynucleotide of claim 27 or 28 under stringent conditions comprising washing at 20-25°C for 5-10 minutes with 2xSSC buffer containing 0.1 % SDS and a subsequent washing with a buffer of 0.1 x SSC buffer with 0.1 % SDS, at a temperature of 64°C.

30(currently amended). An isolated polynucleotide, which hybridizes with an oligonucleotide of claim 29 under stringent conditions, comprising washing at 20-25°C for 5-10 minutes with 2xSSC buffer containing 0.1 % SDS and a subsequent washing with a buffer of 0.1 x SSC buffer containing 0.1 % SDS, at a temperature of 64°C, and encodes ~~codes~~ for a gene product of microorganisms of the *Trypanosoma* genus.

31(currently amended). An isolated polypeptide, which is encoded ~~coded~~ by ~~[[a]]~~ the isolated polynucleotide of claim 27 or 28, which comprises a nucleic acid sequence

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of claim 27.

32(currently amended). An isolated trans-sialidase obtainable from *Trypanosoma congoense*, characterized by one of the following amino acid part sequences:
TDTVKYSTDGGRTWKREVIIPNGR (pos. 1 to 25 of SEQ ID NO: 2) or
FRIPSLVEIDGVLIAFDTRYLRASDSSLI (pos. 1 to 30 of SEQ ID NO: 4).

33(currently amended). The isolated trans-sialidase 1-(TS1) of claim 32, characterized by at least one of the following characteristics:

<u>Nucleotide sequence</u>	<u>SEQ ID NO: 1</u>
Amino acid sequence	<u>comprising</u> SEQ ID NO: 2
Temperature optimum	30-40°C
pH optimum	pH 6.5-8.5
Isoelectric point	pH 4-5
Molecular weight, native	400-600 kDa
Molecular weight in the reducing SDS page	90 kDa

34(currently amended). The isolated trans-sialidase 2-(TS2) of claim 32, characterized by at least one of the following characteristics:

<u>Nucleotide sequence</u>	<u>SEQ ID NO: 3</u>
Amino acid sequence	<u>comprising</u> SEQ ID NO: 4
Temperature optimum	30-40°C
pH optimum	pH 6.5-8.5
Isoelectric point	pH 5-6
Molecular weight, native	120-180 kDa
Molecular weight in the reducing SDS page	90 kDa

35(currently amended). The isolated polynucleotide polynucleotides and trans-

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sialidases of claim 27, isolated from the *Trypanosoma congolense* organism.

36(canceled).

37(currently amended). A trans-sialidase of either ~~claims~~ claim 33 or 34, the amino acid sequence or part sequence of which has a sequence identity of at least 50 % or at least 60 %, in particular at least 65 % or at least 70 %, such as eg. 75 %, 80%, 85 %, 90 %, 95 %, 98 % or 99% to the corresponding amino acid sequence or part sequence of SEQ ID NO: 2 or 4, ~~calculated according to the algorithm of Pearson and Lipman, Proc. Natl. Acad. Sci. (USA) 85(8), 1988, 2444-2448~~; or which contains one or more deletions, additions, substitutions or inversions of an individual or of several amino acid residues or shows a changed glycosylation pattern; whereby the capability of catalysis of the transfer of sialic acids from a donor to an acceptor is maintained.

38(currently amended). [[A]] An isolated nucleotide sequence, encoding a trans-sialidase of claim 32.

39(currently amended). An expression cassette, comprising, ~~in—operative connection—operatively linked to~~ with at least one regulative nucleic acid sequence, a nucleic acid sequence of claim 38.

40(previously presented). A recombinant vector, comprising at least one expression cassette of claim 39.

41(previously presented). Prokaryotic or eucaryotic host, transformed with at least one vector of claim 40.

42(previously presented). A method for the enzymatic sialylation of an acceptor molecule, characterized in that the acceptor molecule is incubated with a donor

containing sialic acid residues in the presence of an enzyme of claim 31, and the sialylated acceptor is isolated.

43(previously presented). The method of claim 42, characterized by at least one more of the following properties:

- a) the donor is selected from the group consisting of sialic acids bonded to oligosaccharides, polysaccharides, polysialic acids, glycoproteins and glycolipids.
- b) the acceptor is selected from the group consisting of polymers containing β -galactose, such as β -galactooligosaccharides, lactitol, lactobionic acid, methyl- β -lactoside, acetyllactosamines, galactopyranosides, trans-galactooligosaccharides, polygalactose and other glycoconjugates with terminally bonded β (1-3) or β (1-4) galactose or galactose.

44(canceled).

45(currently amended). A method ~~amethod~~ for the isolation of an enzyme with trans-sialidase activity as defined in claim 32, whereby

- c) *Trypanosoma congolense* ~~is cultivated in a medium,~~ is cultivated in a medium, and
- b) ~~and the desired product is isolated from the culture supernatant by means of ion exchange chromatography with the help of a salt gradient~~ the desired product is isolated from the culture supernatant by means of ion exchange chromatography by applying a salt gradient.

46(previously presented). The method of claim 45, additionally comprising isoelectric focussing, gel filtration, affinity chromatography and/or protein precipitation.

47(canceled).

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48(currently amended). A foodstuff or food additive comprising containing an effective amount of the isolate of claim 32.